Measuring and Modeling Epidermal Homeostasis on the Cellular Level

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Introduction The systematic analysis and modeling of as large and complex biological networks as possible is the subject of Systems-Biology. Despite the promising future of this discipline in general [1, 2] its modeling side still lacks a clear link to medically relevant applications. It is a matter of current discussions in how far larger systems can be modeled using the toolbox of non-linear dynamics [3, 4]. The complexity of a biological system rises exponentially with the number of its network components. Therefore building models of tissues poses an interesting challenge for research in systems biology. For medical bioinformatics it will become crucial to measure and model tissue structures to gain clinical relevance [5]. This goal will be hard to achieve if the kinetic modeling of intracellular biochemical reactions is chosen as the relevant model layer of abstraction. We here present an alternative approach which concentrates on modeling epidermal homeostasis on the cellular level. This means that cellular proliferation and differentiation are described at a higher level of abstraction compared to direct modeling of biochemical reactions. Modeling the tissue on this higher level of abstraction has two major advantages. Firstly, this approach is relatively close to a clinical application area as the morphological analysis of tissue is the prevailing criteria for defining and diagnosing diseases. Secondly, modeling tissue homeostasis is concerned with rather slow biological processes. An average regeneration of human epidermis takes 20-25 days while signal transduction processes are executed in milli- and microseconds [6]. Slow processes have the advantage of opening the possibility of a semi-quantitative or qualitative modeling. Concluding, we hypothesize that it may be suitable to model tissues quantitatively at the highest level of abstraction and qualitatively at the level of individual cells. This also fits with the observation that the most biological knowledge published in literature today describes intracellular biological phenomena in a rather qualitative way. The question arises how an integrated approach of modeling tissues in such a way and verifying this model against biological experiments could look like. In our contribution we show early results from this approach. First, we demonstrate our novel experimental technique based on immunohistologically stained serial tissue sections yielding quantitative functional and spatial profiles of biomarkers. These profiles (Holo-Charts) describe key protein biomarker concentrations during epidermal homeostasis in epidermal tissue. Secondly the gained profiles are then used as an experimental data basis for computational modeling of epidermal homeostasis. It is shown how qualitative cellular differentiation programs produce biomarker profiles for the whole tissue which resemble those experimentally gained.

Material and Methods

Generation of Holo-Charts: Human epidermal tissue was obtained from healthy patients with their informed consent according to the Helsinki Declaration, and the protocol was approved by the institutional ethic committee. Serial frozen sections of the samples were obtained preceding freezing of the epidermal tissue in liquid nitrogen vapor. The slides were triple stained using indirect immunofluoresence (IIF). Triple staining was comprising of the detection of the respective marker under study together with extracellular matrix collagen type-I, as well as a counterstain of cell nuclei (DAPI). IIF was performed according to previous protocols [7], with foregoing optimization of the working dilutions of primary and secondary antibodies applied to the skin specimens. For image analysis of the tissue slides software was developed using MATLAB 7.1 including the image processing toolbox. Holo-Charts have been defined according to our central motivation of profiling the epithelial differentiation process throughout the tissue. Therefore, they display the normalized marker intensity of a local tissue area in relation to the area´s quantitative state of differentiation. We define the state of differentiation of an area of cells in the epithelial tissue to be equal to the area´s average declining intensity of nuclear staining (DAPI).

Computational Tissue Modeling: As a general modeling approach we chose a spatial multi-agent system in which each cell of the epidermal tissue is represented as an individual agent [8]. The multi-agent system consists of a biomechanical and a biochemical component. The biomechanical component reflects the spatial part of the simulation while the biochemical component controls each participating cell's internal program. The individual agents cannot move by their own force (active cell migration) but can only be moved if necessary to minimize structural forces inside the tissue. As a simple biomechanical model for each cell a force linear to the distance of the cells is assumed. Modeling of cellular processes has been done using finite state machines.

Results

We have shown a novel approach in morphological systems to study epidermal homeostasis. This approach leads to an integrated system consisting of computational modeling and experimental validation. Our new proteomics method is able to functionally roughly profile the epithelial differentiation process using key protein biomarkers. The computational simulation is able to reproduce a layered morphology of the human epidermis, while resulting in functional biomarker profiles equivalent to the gained experimental ones.

Discussion

Although systems-biology is still in its infancy *in-silico* tissue models will become important tools for interpreting experimental results and testing hypothesis concerning epithelial homeostasis. These *in-silico* tissue models will require the profiling of combinatorial panels of biomarkers. Such panels have been described qualitatively already decades ago, e.g. alterations in protein expression profiles have been described when studying how retinoic acid improves epidermal morphogenesis [9]. But to our knowledge up till now no quantitative measurement of known epidermal differentiation markers has been published. In current literature qualitative textual descriptions of individual markers like "the studied marker is expressed in the suprabasal layers" prevail in conjunction with immunohistological images. Unfortunately, such a qualitative description is not sufficient for an *in-silico* model of epithelial homeostasis. Another disadvantage of current immunohistology is that is can resolve only very few biomarkers at the same time. Therefore, we developed the here presented extension to conventional immunohistology utilizing computational image analysis. Our experimental method can resolve the combinatorial expression changes of a large set of proteins during homeostasis of stratified epithelia. The multi-agent approach for tissue modeling has been described in detail previously [8] where related works [10-14] have been discussed. Concerning the modeling of the internal cellular differentiation program, we have chosen a rather uncommon way of modeling cellular behaviour using finite state machines. In principle Petri-nets and cellular automata have been used already for a long time for modeling [3]. Nevertheless these approaches use the individual states for representing individual reaction species while transitions between states are set equivalent to reaction rates [15]. We here follow the alternative route of interpreting the states of the state-machine directly as cellular states. This optimally fits our requirement of modeling qualitatively at the cellular level and results in highly efficient fast multi-cellular simulations. Another key argument for using finite-state machines as the underlying cellular modeling paradigm is the fact that most intracellular biochemical descriptions in literature are only of qualitative nature. Finally, we conclude that our top-down approach of a quantitative description of epithelial homeostasis at the tissue level and qualitative finite state models of individual cells together with our newly developed tissue profiling method provides a novel integrated approach for measuring and modeling epithelial tissue.

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Literatur

[1] Hood, L., et al., *Systems biology and new technologies enable predictive and preventative medicine.* Science, 2004. **306**(5696): p. 640-3
[2] Kitano, H., *Computational systems biology.* Nature, 2002. **420**: p. 206-210
[3] Hofestadt, R. and S. Thelen, *Quantitative modeling of biochemical networks.* In Silico Biol, 1998. **1**(1): p. 39-53
[4] Sauro, H.M., et al., *Next generation simulation tools: the Systems Biology Workbench and BioSPICE integration.* Omics, 2003. **7**(4): p. 355-72

[5] Grabe, N. and K. Neuber, *Morphologische Systembiologie.* Bioforum, 2005(12)
[6] Sneyd, J., J. Keizer, and M.J. Sanderson, *Mechanisms of calcium oscillations and waves: a quantitative analysis.* Faseb J, 1995. **9**(14): p. 1463-72
[7] Tomakidi, P., et al., *Discriminating expression of differentiation markers evolves in transplants of benign and malignant human skin keratinocytes through stromal interactions.* J Pathol, 2003. **200**(3): p. 298-307
[8] Grabe, N. and K. Neuber, *A multicellular systems biology model predicts epidermal morphology, kinetics and Ca2+ flow.* Bioinformatics, 2005. **21**(17): p. 3541-7
[9] Asselineau, D., et al., *Retinoic acid improves epidermal morphogenesis.* Dev Biol, 1989. **133**(2): p. 322-35
[10] Galle, J., M. Loeffler, and D. Drasdo, *Modeling the effect of deregulated proliferation and apoptosis on the growth dynamics of epithelial cell populations in vitro.* Biophys J, 2005. **88**(1): p. 62-75
[11] Meineke, F.A., C.S. Potten, and M. Loeffler, *Cell migration and organization in the intestinal crypt using a lattice free model.* Cell Proliferation 2001, 2001. **34**: p. 253-266
[12] Noble, D., *Systems biology and the heart.* Biosystems, 2006. **83**(2-3): p. 75-80
[13] Rashbass, J., D. Stekel, and E.D. Williams, *The use of a computer model to simulate epithelial pathologies.* J Pathol, 1996. **179**(3): p. 333-9
[14] Walker, D., et al., *The Epitheliome: agent based modelling of the social behaviour of cells.* BioSystems, 2004. **76**: p. 89-100
[15] Reddy, V.N., M.N. Liebman, and M.L. Mavrovouniotis, *Qualitative analysis of biochemical reaction systems.* Comput Biol Med, 1996. **26**(1): p. 9-24